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2015

Chandrasekharan, P., Yang, C.-T., Nasrallah, F. A., Tay, H. C., Chuang, K.-H., & Robins, E. G. (2015). Pharmacokinetics of Gd(DO3A-Lys) and MR imaging studies in an orthotopic U87MG glioma tumor model. *Contrast Media & Molecular Imaging*, 10(3), 237-244.

<https://hdl.handle.net/10356/82758>

<https://doi.org/10.1002/cmml.1634>

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[<http://dx.doi.org/10.1002/cmml.1634>].

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Journal:	<i>Contrast Media and Molecular Imaging</i>
Manuscript ID:	CMMI-14-0014.R1
Wiley - Manuscript type:	Full Paper
Date Submitted by the Author:	n/a
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Keyword:	gadolinium chelate , pharmacokinetics, tumor imaging, magnetic resonance imaging , orthotopic glioma model

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**Pharmacokinetics of Gd(DO3A-Lys) and MR Imaging Studies in an Orthotopic
U87MG Glioma Tumor Model**

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ABSTRACT:

Pharmacokinetics of Gd(DO3A-Lys), a macrocyclic gadolinium based MRI contrast agent functionalized with a lysine derivative, was studied in Wistar rats. Kinetic data was fitted using a two compartment model and revealed Gd(DO3A-Lys) to have a distribution half-life, $t_{1/2}(\alpha)$, of 1.3 min, an elimination half-life, $t_{1/2}(\beta)$, of 24.9 min and a large volume of distribution, V_D , of 0.49 L/kg indicative of the agent being able to rapidly distribute into tissues and organs. Contrast enhanced magnetic resonance angiography (CE-MRA) in an orthotopic U87MG glioma mouse model demonstrated considerable enhancement of both the tumor and surrounding vasculature after intravenous administration of Gd(DO3A-Lys). Applying dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) in the glioma of different sizes further showed distinct uptake characteristics and patterns of enhancement, which suggests the potential for differentiating changes at different stages of tumor growth. Our results indicate that Gd(DO3A-Lys) could be a promising candidate for glioma MR imaging.

Keywords: gadolinium chelate • magnetic resonance imaging (MRI) • pharmacokinetics • contrast agent • tumor diagnosis • orthotopic glioma model

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6 **1. Introduction**
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9 Gadolinium (Gd(III)) based MRI contrast agents have been widely used in clinical
10 practice for the detection and diagnosis of cancer lesions [1-3], atherosclerotic plaques [4],
11 osteoarthritis [5] and vascular occlusions [6]. Contrast agents have been found to be
12 particularly useful for the grading of invasive tumors and the assessment of treatment
13 efficacy. MR perfusion imaging of tumors using contrast agents, where the contrast agent
14 uptake is dynamically monitored, enables parameters such as blood volume, blood flow,
15 vessel permeability and extracellular volume fraction to be calculated [7, 8]. Through
16 analysis of the observed signal enhancement, MR perfusion imaging is able to delineate
17 between malignant and non-malignant tumors based on the uptake characteristics of the
18 contrast agent [1, 9]. Most low molecular weight gadolinium based MRI contrast agents are
19 gadolinium chelates of DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic) or
20 DTPA (diethylene-triamine-penta acetic acid) [10-12], however, more recently modified
21 derivatives of these two chelators have been developed. Functionalization with biologically
22 active compounds such as amino acids, polypeptides and other cell surface specific ligands
23 has enable the development of targeted MR imaging agents [13,14]. In particular,
24 macromolecular DTPA derivatives conjugated to synthetic and natural polymers such as
25 polylysine, serum albumin and polysaccharide dextran have been evaluated for tumor and
26 vascular integrity imaging [15-18]. Despite the advances made, many of these
27 macromolecular gadolinium agents often exhibit long kidney retention [16], poor clearance
28 characteristics [18] and are unstable under heat sterilization [19]. In order to address some of
29 the shortcomings of macromolecular agents, low molecular weight tri- and penta-lysine
30 peptides with DTPA derivatives have been synthesized and their pharmacokinetic and
31 biodistribution characteristics evaluated [20]. The half-life in blood of these new polylysine
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derivatives, as determined in rats, is two-fold longer than that of Gd-DTPA. Their moderate retention in blood and their higher stability suggested these new polylysine derivatives could be potential intravascular agents for MR angiography [20].

Amino acids, the building blocks of proteins, play important functions in cells and metabolism. Lysine is an essential amino acid, and it is a vital component in all the proteins required by the human body. As early as 1955, lysine was understood to have an increased accumulation in cancerous cells compared to normal cells [21]. We have developed two contrast agents based on Gd-DO3A chelates conjugated to amino acid building blocks derived from glutamate and lysine [22]. Our initial *in vivo* MR imaging studies of these two contrast agents in Wistar rats showed significant arterial signal enhancement post intravenous injection. Gd(DO3A-Lys) (Fig.1) exhibited a longer blood half-life by comparison with the glutamate derivative and further evaluation of Gd(DO3A-Lys) demonstrated high accumulation in visceral organs including gastrointestinal tract, high uptake in HT1080 and U87MG subcutaneous xenografts as well as negligible toxicity at relatively high dose (1.0 mmol/kg) [23]. Our preliminary studies indicated the potential of Gd(DO3A-Lys) as an intravascular agent for tumor angiography [23]. These preliminary results gave us the impetus to undertake an *in vivo* pharmacokinetic study of Gd(DO3A-Lys). In addition, to build on our initial preclinical data, a more clinically relevant orthotopic glioma model of U87MG was used to evaluate Gd(DO3A-Lys) as an intravascular contrast agent for the dynamic contrast enhanced MRI (DCE-MRI) study of tumor growth and contrast enhanced magnetic resonance angiography (CE-MRA).

2. Results and discussion

2.1. Pharmacokinetic study

Our initial pharmacokinetic evaluation of Gd(DO3A-Lys) was conducted using Wistar rats, at doses of 0.1, 0.2 and 0.4 mmol/kg. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used for the rapid quantification of Gd(DO3A-Lys) in blood with a detection limit of 0.01mg/L. Blood samples (0.2 mL aliquots) were collected at regular time intervals up to 90 min post injection of Gd(DO3A-Lys). The pharmacokinetic data obtained for Gd(DO3A-Lys) was fitted to the two-compartment model illustrated in Figure 2. The central compartment (compartment 1) consisting of blood and highly perfused tissues (e.g., kidney and liver) whereas the peripheral compartment (compartment 2) represents poorly perfused tissues including muscles, fat and lean tissues. K_{12} , K_{21} and K_{10} represent the transfer rate constants between different compartments (Fig 2). The pharmacokinetic data was fitted using a biexponential equation using Kinetica[®] software. The macroconstants (hybrid constants) obtained were used to calculate the microconstants as summarized in Table 1. At a dose of 0.1 mmol/kg, corresponding to the typical clinical dose for gadolinium based contrast agents, the distribution half-life $t_{1/2}(\alpha)$ for Gd(DO3A-Lys) is 1.3 min. The short distribution half-life suggests rapid distribution of contrast agent into well-perfused tissues and organs ($K_{12} \gg K_{21}$), similar characteristics were observed at higher administered doses (0.2 and 0.4 mmol/kg). Figure 3 shows the time-concentration curve in rat blood (mg/L) after a single bolus intravenous administration of 0.1, 0.2 and 0.4 mmol/kg Gd(DO3A-Lys). After the distribution phase, the concentration of Gd(DO3A-Lys) in blood reaches pseudo-equilibrium preceding the slow and steady elimination phase. The elimination half-life $t_{1/2}(\beta)$ of Gd(DO3A-Lys) at a dose of 0.1 mmol/kg is 24.9 min. The long elimination half-life suggests that Gd(DO3A-Lys) is either continuously circulated in the organs or is slowly excreted from renal and hepatobiliary systems ($K_{10} \leq K_{12}$). The volume of distribution for Gd(DO3A-Lys) was calculated to be 0.49 ± 0.08 L/kg, which is indicative of extracellular and intracellular distribution (V_D larger than 0.30 L/kg) [24]. At 0.1 mmol/kg

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3 injected dose, the clearance of Gd(DO3A-Lys) was 0.068 ± 0.005 L/min/kg. Therefore, the
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5 reduction in blood concentration of Gd(DO3A-Lys) can be attributed to the combined effect
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7 of elimination and perfusion into the peripheral compartment. Nevertheless, the blood
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9 concentration of Gd(DO3A-Lys) at later time points was in parts per million levels which is
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11 still within the detection capability of MRI.
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15 Table 2 shows a comparison of the pharmacokinetic parameters obtained for Gd(DO3A-
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17 Lys) and Dotarem[®] in Wistar rats at the same concentration (0.1 mmol/kg). Gd(DO3A-Lys)
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19 has a considerably longer elimination half-life in rats (24.9 min) when compared to that of
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21 Dotarem[®] (18.0 min) [25-27]. The volume of distribution for Gd(DO3A-Lys) (0.49 L/kg) is
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23 almost 2.5 times that of Dotarem[®] (0.20 L/kg) and the elimination half-life of Gd(DO3A-
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25 Lys) is significantly longer than has previously been observed for other agents such as
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27 Gadovist[®] (13-18 min in rats) [24]. The pharmacokinetic profile of Gd(DO3A-Lys) is similar
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29 to that observed for the DTPA tri- and penta-lysine derivatives [20], which demonstrated an
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31 increased V_D and $t_{1/2}(\text{beta})$ when compared to the parent Gd-DTPA. Similarly, a large V_D and
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33 comparable $t_{1/2}(\text{beta})$ was also observed for Vasovist[®] in rats but at a lower administered dose
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35 of 0.03 mmol/kg [27]. The favourable distribution characteristics of Gd(DO3A-Lys)
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37 prompted us to further evaluate it as intravascular contrast agent for CE-MRA studies using
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39 an orthotopic glioma model.
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44 2.2. *In Vivo MR imaging studies in an orthotopic glioma model*

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47 An orthotopic glioma model was chosen for the *in vivo* imaging evaluation of
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49 Gd(DO3A-Lys). The orthotopic model was developed by stereotactic injection of U87MG
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51 human glioblastoma cells into the brain. The model recapitulates the original tumor
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53 microenvironment as the tumor cells are directly implanted into the organ of origin.
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55 Moreover, the U87MG glioma cells, exhibit upregulated VEGF/VPF expression and show a
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high degree of angiogenesis which is ideal for the study and development CE-MRA agents [28]. The tumor growth was monitored at regular intervals using T₂-weighted MR images for up to 24 days (data not shown, tumor volume = 2.0 ± 0.4 mm³). Figure 4 shows the T₁-weighted images of a high grade U87MG glioma in mouse brain before and after Gd(DO3A-Lys) administration. After administration, the contrast agent clearly demarcates the tumor with higher signal intensity than the surrounding tissue and is retained in the tumor for at least 25 min. Signal enhancement in the brain, carotid artery and tumor during the first 10 min after contrast agent administration is shown in Figure 5. The signal enhancement in the blood vessel had a rapid distribution phase followed by a gradual and slow clearance phase. The signal enhancement in the tumor showed similar rapid distribution phase but no obvious clearance phase over 10 min. The long retention of Gd(DO3A-Lys) in the tumor could be due to leaky nature of the newly formed vessels and the disruption of blood-brain barrier (BBB).

Contrast enhanced MR angiography (CE-MRA) is a powerful tool in differentiating the arterial and venous blood vessels *in vivo* [29]. Intravenously administered contrast agent reaches the artery first from the heart before reaching the venous circulation followed by cardiovascular re-circulation. Contrast agents for imaging tumor vasculature are well developed for use in computed tomography (CT) [30] and MRI [31]. Figure 6 shows the post-processed maximum intensity projection images (MIP) of CE-MRA obtained over 10 min during Gd(DO3A-Lys) enhancement (injected at 1 min ± 18 sec). Figure 6B shows considerable enhancement of major arterial blood vessels immediately after bolus injection. The signal from the tumor (yellow arrow) increased in intensity compared to the contralateral side at 1 min 9 sec (Fig. 6C). With time, the signal of major vessels started to drop whilst the enhancement in the tumor and associated vessels (yellow arrow) were visible at 2 min 14 sec (Fig. 6D). At 6 min 44 sec (Fig. 6E), the tumor appears brightest with highest contrast compared to the surrounding tissue. In particular, a bifurcated vessel associated with

the tumor could be clearly observed. The highest tumor to non-tumor contrast was observed at 10 min 42 sec as shown in Figure 6F, where the enhancement of all other vessels has decreased and is most likely as a result of the enhanced permeation and retention of Gd(DO3A-Lys).

Gd(DO3A-Lys) was further evaluated using DCE-MRI to investigate potential differences in uptake characteristics and patterns of tumor enhancement related to changes in tumor homogeneity at different stages of tumor growth. Two different time points were chosen (day 12 and day 17 post-inoculation) to reflect the early and late stages of tumor development. T₁-weighted images at day 12 and day 17 of tumor growth both pre- and post-contrast (0.1 mmol/kg administration of Gd(DO3A-Lys)) are shown in Figure 7. The tumor volumes measured at days 12 and 17 were 0.68 ± 0.14 and 1.46 ± 0.29 mm³, respectively. At day 17, images showed a higher peak signal enhancement but also a greater change in the signal-to-noise ratio (SNR) by comparison to that observed at day 12. Figure 8 shows the pattern of Gd(DO3A-Lys) uptake kinetics at day 12 and day 17. At the earlier timepoint, the rapid uptake phase of Gd(DO3A-Lys) was followed by a plateau phase, however, at the later timepoint, the rapid uptake phase was followed by a washout phase. Table 3 summarizes the size of the tumor and the rate of contrast agent uptake at days 12 and 17 in three different mice. There was good correlation ($r = 0.98$ at day 12 and $r = 0.5$ at day 17; $p < 0.05$) between the tumor volume and the contrast agent uptake rate. Since the uptake rate is proportional to the tumor blood flow and the higher blood flow is indicative of angiogenesis, this may potentially be used for assisting the grading of the tumor [32]. The clearance of tracer is related to the permeability surface area [33]. The faster clearance at day 17 suggests a higher vascular permeability that may correspond to the leaky nature of fast growing but immature blood vessels in the tumor.

3. Conclusion

We have undertaken the initial pharmacokinetic characterisation of Gd(DO3A-Lys) in Wistar rats. Gd(DO3A-Lys) distributes into tissues and organs quickly once administered intravenously (V_D 0.49 ± 0.08 L/kg) and demonstrates a relatively long elimination half-life ($t_{1/2}(\text{beta})$ 24.9 min). *In vivo* MR imaging studies of Gd(DO3A-Lys) in an orthotopic U87MG glioma model have demonstrated the application of Gd(DO3A-Lys) as a potential agent for both CE-MRA and DCE-MRI. CE-MRA studies demonstrated that Gd(DO3A-Lys) tumor signal enhancement can readily differentiate the tumor and associated vasculature from the surrounding tissues. Analysis of the perfusion kinetics of Gd(DO3A-Lys) demonstrated differential profiles can be observed at different stages of tumor growth. Thus, Gd(DO3A-Lys) has the potential to be used as an agent to differentiate changes in tumor microvasculature during angiogenesis and tumor growth. Further pharmacokinetics and ADME studies on other animal species are necessary to complete pre-clinical trials of Gd(DO3A-Lys).

4. Experimental section

4.1 Materials and methods

All solvents and reagents were used as received or purified if required, using standard methods. Gd(DO3A-Lys) (Fig. 1) was synthesized by Beijing PharmaSciences Co. Ltd (Beijing, China) according to procedures from previous study [23]. ICP-AES was performed on Optima 5300DV system (PerkinElmer, USA). Pharmacokinetic study was conducted on male Wistar rats (bodyweight 350 ± 50 g, Biological Resource Center, A*STAR). *In vivo*

MR imaging was conducted on female SCID mice (bodyweight 20 ± 2 g, Biological Resource Center, A*STAR). The animal were cared for under an approved protocol (IACUC Number: 100591) and the guidelines of the Responsible Care and Use of Laboratory Animal Committee, Biological Resource Center, A*STAR.

4.2. Pharmacokinetics study

Male Wistar rats (10 - 12 weeks, weights range 300 - 400 g) were fed *ad libitum* with normal rodent diet and maintained in a room with a 12 h light/dark cycle at 20 ± 2 °C. After one week acclimatization period, the animals were randomly divided into three dosage groups ($n = 5$ per group). Gd(DO3A-Lys) (181.45 mg/mL) was dissolved in 0.9% NaCl saline for injection. The solutions were filtered sterilized before injected into the rats via a tail vein. The injection volume was 0.46, 0.92 and 1.84 mL/kg making dosages of 0.1, 0.2 and 0.4 mmol/kg, respectively. These three doses have been chosen as 0.1 mmol/kg is the dose that we used for diagnostic angiography. The other two dosed are two and four times of this concentration, respectively.

For pharmacokinetics, a 0.2 mL aliquot of blood sample was collected into 2 ml tube from the rat tail vein at 0, 2, 3, 4, 6, 10, 20, 40, 90 min time points after intravenous administration of Gd(DO3A-Lys). Equal volume (0.2 ml) of 0.9% normal saline was injected to compensate the volume loss after each blood collection. Blood samples were transferred into the microwave digestion liner tubes. The samples were then left overnight in the tubes together with nitric acid. Microwave digestion was done the next morning. After microwave digestion, the samples were made up to 30 ml using ultrapure water and Gd(III) concentration analyzed by ICP-AES. The two-compartmental fit was done considering the compartments of

distribution of the contrast agent as shown in Fig. 2. The concentration-time profile was fitted by the following equation using WinNonlin 5.2[®] and the kinetic parameters were estimated:

$$C_B(t) = Ae^{-\alpha t} + Be^{-\beta t}$$

C(t): plasma drug concentration; A and B: hybrid constants; t: time

$$\alpha = \frac{(k_{12}+k_{21}+k_{10})+\sqrt{(k_{12}+k_{21}+k_{10})^2-4k_{21}k_{12}}}{2} \quad \beta = \frac{(k_{12}+k_{21}+k_{10})-\sqrt{(k_{12}+k_{21}+k_{10})^2-4k_{21}k_{12}}}{2}$$

ICP-AES was used to analyze Gd(DO3A-Lys) concentrations in the blood samples. The plasma flow was set as 15 L/min, auxiliary flow 0.2 L/min, nebuliser flow 0.8 L/min and pump rate 1 ml/min. Both instrument stabilization delay and sample uptake delay were set as 8 s. Each sample was analyzed in triplicate. The detection limit of the instrument were established based on 10 readings each from a standard solution of ultrapure water, blank rat's blood with no spiking of Gd(DO3A-Lys) and rat's blood spiked with 0.1 mg/kg of Gd(DO3A-Lys). Limit of blank (LoB) was calculated based on Mean_{blank} ± SD_{blank}. Recovery was calculated by spiking rat's blood with a range of known amount of Gd(DO3A-Lys) and analyzed for 10 times. Recovery was calculated using equation below:

$$\text{Recovery (\%)} = [\text{Mean (spiked reading)}/\text{Concentration of analyte spiked}] * 100\%.$$

4.3. Orthotopic Glioma tumor model and in vivo MR imaging

Orthotopic glioma model was developed on female SCID mice (20 ± 2 g, 6 weeks old). 2 x 10⁵ human glioblastoma U87MG cells in 2 µL of 1x PBS was stereotaxically injected into the mouse brain using a Hamilton[®] syringe at 1.6 mm on the right of midline through bregma, and 3.5 mm into the parenchymal tissue where the brain tissue is homogenous. The tumor was allowed to grow, and was monitored regularly with a preclinical 7 Tesla MRI scanner (ClinScan, Bruker BioSpin, Germany). Four-channel mouse brain array receive coil

was used along with a 72 mm transmit volume coil. T₁-weighted gradient echo sequence with TR/TE = 400/2.6 ms, flip angle = 90⁰, and 2 averages were used to acquire 18 of 1 mm slices, with a pixel resolution of 0.07 mm x 0.07 mm in transverse direction at different times before and after injection of the contrast agent.

Dynamic angiograms were acquired using a T₁-weighted 3D FLASH sequence with TR/TE = 5/1.14 ms and flip angle of 60⁰. 52 slices in the coronal direction were taken with a slice thickness of 0.3 mm, and pixel resolution of 0.08 mm x 0.08 mm. Temporal resolution was 8.2 sec. 80 dynamic images were acquired over 11 min. Gd(DO3A-Lys) was administered at first min ($\pm$ 0.3 min) of MRI acquisition at a dose of 0.1 mmol/kg of animal body weight. Angiograms were obtained by subtracting post-contrast images from pre-contrast images after linear registration (ImageJ) and displayed as maximum intensity projection (MIP). MIPs were created using slice selective volumes using ImageJ. Tracer signal enhancement curve in different tissues was determined by drawing volume of interest (VOI). The signal was normalized by the noise from background to derive the signal-to-noise ratio (SNR). The change in signal was determined with respect to the baseline scans before contrast agent administration. Kinetic curve analysis was done using MIPAV 5.3.

Acknowledgments

This work was supported by COT Grant ETPL/10-S10COT-006 of A*STAR (Agency for Science, Technology and Research); Singapore Bioimaging Consortium Intramural Funding of the Biomedical Research Council, Singapore. Authors thank Kai Yang from SGMed International Pte Ltd for helpful discussion.

References

[1] Neubauer H, Li M, Kuehne-Heid R, Schneider A, Kaiser WA. High grade and non-high grade ductal carcinoma in situ on dynamic MR mammography: characteristic findings for signal increase and morphological pattern of enhancement. *Br J Radiol* 2003; 76:3-12.

[2] Gandhi SN, Brown MA, Wong JG, Aguirre DA, Sirlin CB. MR contrast agents for liver imaging: what, when, how. *Radiographics* 2006; 26:1621-36.

[3] Zhou Z, Lu ZR. Gadolinium-based contrast agents for magnetic resonance cancer imaging. *WIREs Nanomed Nanobiotechnol* 2013; 5:1-18.

[4] Yuan C, Kerwin WS, Ferguson MS, Polissar N, Zhang S, Cai J, et al. Contrast-enhanced high resolution MRI for atherosclerotic carotid artery tissue characterization. *J Magn Reson Imaging* 2002;15:62-7.

[5] Kim SD, Jessel R, Zurakowski D, Millis MB, Kim YJ. Anterior delayed gadolinium-enhanced MRI of cartilage values predict joint failure after periacetabular osteotomy. *Clin Orthop Relat Res* 2012; 470:3332-41.

[6] Lauffer RB, Parmelee DJ, Dunham SU, Ouellet HS, Dolan RP, Witte S, et al. MS-325: albumin-targeted contrast agent for MR angiography. *Radiology* 1998; 207:529-38.

[7] Pauliah M, Saxena V, Haris M, Husain N, Rathore RK, Gupta RK. Improved T(1)-weighted dynamic contrast-enhanced MRI to probe microvascularity and heterogeneity of human glioma. *Magn Reson Imaging* 2007; 25:1292-9.

- [8] Li SP, Padhani AR, Taylor NJ, Beresford MJ, Ah-See ML, Stirling JJ, et al. Vascular characterisation of triple negative breast carcinomas using dynamic MRI. *Eur Radiol* 2011; 21:1364-73.
- [9] Facius M, Renz DM, Neubauer H, Bottcher J, Gajda M, Camara O, et al. Characteristics of ductal carcinoma in situ in magnetic resonance imaging. *Clin Imaging* 2007; 31:394-400.
- [10] Caravan P, Ellison JJ, McMurry TJ, Lauffer RB, Gadolinium(III) chelates as MRI contrast agents: structure, dynamics, and applications *Chem Rev* 1999, 99, 2293-2352.
- [11] Carravan P. Strategies for increasing the sensitivity of gadolinium based MRI contrast agents. *Chem Soc Rev* 2006, 35, 512-523.
- [12] Terreno E, Castelli DD, Viale A, Aime S. Challenges for molecular magnetic resonance imaging. *Chem Rev* 2010, 110, 3019-3042.
- [13] Breeman WA, de Blois E, Sze Chan H, Konijnenberg M, Kwekkeboom DJ, Krenning EP. (68)Ga-labeled DOTA-peptides and (68)Ga-labeled radiopharmaceuticals for positron emission tomography: current status of research, clinical applications, and future perspectives. *Semin Nucl Med* 2011; 41:314-21.
- [14] Chandrasekharan P, Yong CX, Poh Z, He T, He Z, Liu S, et al. Gadolinium chelate with DO3A conjugated 2-(diphenylphosphoryl)-ethyldiphenylphosphonium cation as potential tumor-selective MRI contrast agent. *Biomaterials* 2012; 33:9225-31.
- [15] Grandin C, Van Beers BE, Demeure R, Goudemant JF, Mottet I, Pringot, J. Comparison of Gadolinium-DTPA and polylysine-gadolinium-DTPA-enhanced magnetic resonance imaging of hepatocarcinoma in the rat. *Invest Radiol* 1995; 30:572-81.

- [16] Schuhmann-Giampieri G, Schmitt-Willich, H, Frenzel T, Press WR, Weinmann HJ. *In vivo* and *in vitro* evaluation of Gd-DTPA-polylysine as a macromolecular contrast agent for magnetic resonance imaging. *Invest Radiol* 1991; 26:969-74.
- [17] Parac-Vogt TN, Kimpe K, Laurent S, Vander Elst L, Burtea C, Chen F, Muller RN, Ni Y, Verbruggen A, Binnemans K. Synthesis, characterization and pharmacokinetic evaluation of a potential MRI contrast agent containing two paramagnetic centers with albumin binding affinity. *Chem Eur J* 2005, 11:3077-3086.
- [18] Wang SC, Wikström MG, White DL, Klaveness J, Holtz E, Rongved, P, Moseley ME, Brasch RC. Evaluation of Gd-DTPA-labeled dextran as an intravascular MR contrast agent: imaging characteristics in normal rat tissue. *Radiology* 1990, 175:483-488.
- [19] Schmiedl U, Ogan M, Paajanen H, Marotti M, Crooks LE, Brito AC, Brasch RC. Albumin labeled with Gd-DTPA as an intravascular, blood-pool enhancing agent for MR imaging: biodistribution and imaging studies. *Radiology* 1987; 162:205-210.
- [20] Laurent S, Burtea C, Vander Elst L, Muller RN. Synthesis and characterization of new low-molecular-weight lysine-conjugated Gd-DTPA contrast agents. *Contrast Media Mol Imaging* 2011, 6:229-235.
- [21] Wiseman G, Ghadially FN. Studies in amino-acid uptake by RD3 sarcoma cell suspensions in vitro. *Br J Cancer*. 1955;9:480-5.
- [22] Kittigowittana K, Yang CT, Cheah WC, Chuang KH, Tuang CY, Chang YT, et al. Development of intravascular contrast agents for MRI using gadolinium chelates. *ChemMedChem* 2011; 6:781-7.

- [23] Yang CT, Chandrasekharan P, He T, Poh Z, Raju A, Chuang KH, et al. An intravascular MRI contrast agent based on Gd(DO3A-Lys) for tumor angiography. *Biomaterials* 2014; 35:327-36.
- [24] Aime S, Caravan P. Biodistribution of gadolinium-based contrast agents, including gadolinium deposition. *J Magn Reson Imaging* 2009; 30:1259-67.
- [25] Weinmann HJ, Brasch RC, Press WR, Wesbey GE. Characteristics of gadolinium-DTPA complex: a potential NMR contrast agent. *Am J Roentgenol* 1984; 142:619-24.
- [26] Bousquet JC, Saini S, Stark DD, Hahn PF, Nigam M, Wittenberg J, et al. Gd-DOTA: characterization of a new paramagnetic complex. *Radiology* 1988; 166:693-8.
- [27] Leiner T, Goyen M, Rohrer M, O SS, (Eds.). Technical aspects of contrast enhanced MRA - First pass and steady state. *Clinical blood pool MR imaging. Volume XVIII: Heidelberg, Germany: Springer Medizin Verlag; 2008. p 16-32.*
- [28] Claffey KP, Robinson GS. Regulation of VEGF/VPF expression in tumor cells: consequences for tumor growth and metastasis. *Cancer Metastasis Rev.* 1996;15:165-76.
- [29] Korosec FR, Frayne R, Grist TM, Mistretta CA. Time-resolved contrast-enhanced 3D MR angiography. *Magn Reson Med* 1996; 36:345-51.
- [30] Ghaghada KB, Badea CT, Karumbaiah L, Fettig N, Bellamkonda RV, Johnson GA, et al. Evaluation of tumor microenvironment in an animal model using a nanoparticle contrast agent in computed tomography imaging. *Acad radiol* 2011; 18:20-30.
- [31] Kobayashi H, Sato N, Kawamoto S, Saga T, Hiraga A, Ishimori T, et al. 3D MR angiography of intratumoral vasculature using a novel macromolecular MR contrast agent. *Magn Reson Med* 2001;46:579-85.

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[32] Cha S. Update on brain tumor imaging: from anatomy to physiology. Am J Neuroradiol 2006; 27:475-87.

[33] Zaharchuk G. Theoretical basis of hemodynamic MR imaging techniques to measure cerebral blood volume, cerebral blood flow, and permeability. Am J Neuroradiol 2007; 28:1850-8.

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Table 1. Major pharmacokinetics parameters analyzed by a two-compartment model of analysis for Gd(DO3A-Lys) in Wistar rats after a single dose intravenous administration

Parameter *	Unit	Dose (mmol/kg)		
		0.1	0.2	0.4
AUC	min*mg/L	1227.4 ± 85.8	3977.3 ± 998.4	6310.0 ± 1338.8
K ₁₀	L/min	0.14 ± 0.03	0.42 ± 0.32	0.14 ± 0.07
K ₁₂	L/min	0.17 ± 0.07	0.48 ± 0.42	0.27 ± 0.19
K ₂₁	L/min	0.044 ± 0.022	0.066 ± 0.004	0.091 ± 0.049
V _D	L/kg	0.49 ± 0.08	0.28 ± 0.34	0.43 ± 0.18
C _{max}	mg/L	171.53 ± 28.64	1462.30 ± 1091.08	868.98 ± 312.11
CL	L/min/kg	0.068 ± 0.005	0.044 ± 0.010	0.051 ± 0.011
MRT	min	35.6 ± 10.1	25.9 ± 12.7	30.1 ± 7.3
V _{ss}	L/kg	2.41 ± 0.52	1.05 ± 0.25	1.59 ± 0.09
A	mg/L	277.6	1035	639.9
B	mg/L	20.33	88.24	132.5
a	L/min	0.5292	1.18	0.4116
b	L/min	0.0278	0.0315	0.0281

*AUC – Area Under the Curve; K₁₀ – elimination rate constant; K₁₂ – rate constant for transfer from central compartment to the peripheral compartment; K₂₁ - rate constant for transfer from peripheral compartment to the central compartment; V_D – Volume of distribution; C_{max} –Maximum blood concentration; CL – Clearance; MRT – mean residence time; V_{ss} – volume at steady state; A,B, a, and b are micro constants from the two compartment rate equation.

Table 2. Comparison of pharmacokinetic parameters of Gd(DO3A-Lys) with Dotarem

PK parameter /Units*	Gd (DO3A-Lys) 0.1 mmol/kg (Rat)	Dotarem 0.1 mmol/kg (Rat) [23]
$t_{1/2}(\alpha)$ min	1.3	3.0
$t_{1/2}(\beta)$ min	24.9	18
V_D (L/kg)	0.49	0.20
CL (L/min/kg)	0.068	0.008
Vss (L/kg)	2.41	-
$AUC_{0-\infty}$ (mmol x minute)	1.49	-

* V_D – Volume of distribution; CL – Clearance; Vss – volume at steady state; AUC – Area Under the Curve

Table 3. Tumor uptake rate at different tumor volume

	DAY 12		DAY 17	
	Tumor Volume mm ³	Rate of tracer uptake [#] [arb.u min ⁻¹]	Tumor Volume mm ³	Rate of tracer uptake [#] [arb.u min ⁻¹]
Mouse 1	0.66	0.3965	1.25	1.5033
Mouse 2	0.83	0.4517	1.80	1.6429
Mouse 3	0.55	0.37872	1.33	0.6934

[#]calculated from the slope of the signal versus time curve at the signal enhancement phase of the contrast agent. $(\frac{SNR_{t2}-SNR_{t1}}{t_2-t_1})$ slope at the signal enhancement phase

Fig. 1. Structure of Gd(DO3A-Lys)

Fig. 2. Compartmentalization for a two-compartment model, with the contrast agent administered as bolus intravenously

Fig. 3. The time-concentration curve in rat blood (mg/L, showing only the positive SD) after a single dose intravenous administration of 0.1, 0.2 and 0.4 mmol/kg Gd(DO3A-Lys)

Fig. 4. Pre- and post-contrast T₁-weighted images. The Gd(DO3A-Lys) shows a considerable uptake in the tumor, resulting from the leaky nature of the tumor vasculature.

Fig. 5. Dynamic contrast enhancement in different tissues. The glioma tumor had a steady uptake followed by signal saturation after administration of Gd(DO3A-Lys) at 1.3 min. The carotid artery supplying blood to the head and neck had a signal increase and a decrease, indicating an in and exit phase of the contrast agent in the blood vessel. The normal brain tissue had no signal enhancement, which indicates that the disruption of blood-brain barrier due to tumor growth is essential for contrast enhancement by exogenous materials that do not cross blood-brain barrier naturally.

Fig. 6. Dynamic angiography of vessel and tumor signal enhancement after intravenous administration of Gd(DO3A-Lys). Images A to F show maximum-intensity projections (MIP) of post-processed images. A, precontrast image; B and C, immediately after contrast agent administration (1 min); D and E, the tumor and associated tumor vessels enhanced by the contrast agent at 2 min and 14 sec and 6 min 44 sec, respectively; F, the angiography image at 10 min and 42 sec (Yellow arrow points to the tumor associated vessels and green arrow points to the tumor).

Fig. 7. T₁-weighted MR images acquired pre and post Gd(DO3A-Lys) administration in three mice with an orthotopic glioma tumor at day 12 and day 17 post tumor implantation.

Fig. 8. Signal enhancement kinetics after Gd(DO3A-Lys) administration in mouse with an orthotopic glioma tumor at day 12 and day 17 post tumor implantation.